

DIFFERENCES IN SPERMATOPHORE AVAILABILITY AMONG OCTOPODID SPECIES (CEPHALOPODA: OCTOPODA)

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ABSTRACT

Female octopuses are thought to copulate with multiple males and store sperm for months prior to spawning, a generalization that has led to hypotheses of strong sperm competition throughout the family. Their diversity, and the difficulty in observing many octopus species in the wild, force indirect tests of this generalization. Male reproductive effort, already high in these generally semelparous animals, is predicted to increase further with sperm competition. Hypothesized functional constraints on the spermatophores that males build to transfer sperm could mean that selection impacts spermatophore number more than morphology. To test whether the number of spermatophores could indicate differences in octopodid biology, the greatest number of spermatophores carried by one individual, and longest spermatophore and mantle lengths were compiled for 74 species. Differences in number were predicted to be independent of differences in specimen availability, related species were predicted to have similar counts, and counts were predicted to be largely independent of spermatophore and mantle length. The median spermatophore count was 24; nearly one-third of the species had 60 or more spermatophores. Of these, 16 were species of *Eledone*, *Octopus* (*Octopus*) or *Abdopus*, supporting the hypothesis that related species share similar spermatophore counts; relationships of the other species are poorly known. Spermatophores of these 24 species tended to be slightly shorter relative to mantle length than in the family as a whole. *Callistoctopus* spp. carry nine or fewer spermatophores, as do deep-sea species of *Bathypolypus*, and the Southern Ocean endemic and deep-sea octopus clade. The number of specimens did not differ between species with fewer or more than the median number. Differences in spermatophore counts among species may offer insight into male response to sperm competition and the unsuspected diversity of reproductive behavior in benthic octopods.

Key words: meristic character, sperm, male reproductive investment, *Abdopus*, *Eledone*, *Octopus* (*Octopus*), *Callistoctopus*.

INTRODUCTION

The race between sperm from two different males to fertilize an egg is termed sperm competition (Birkhead, 2000). Sperm competition may occur in any mating system in which females accept more than one male as a mate. Benthic octopuses of the Octopodidae are one such group. Data available from a very limited number of species indicate that female octopuses copulate with multiple males and store sperm for up to several months; sperm competition has therefore been suggested to be intense (Hanlon & Messenger, 1996; Birkhead, 2000). Further support for this inference derives from microsatellite analysis that found a female of *Graneledone boreopacifica* Nesis, 1982, used sperm from at least two males to fertilize her

egg clutch (Voight & Feldheim, accepted) and that males of *Abdopus aculeatus* (d'Orbigny, 1834) mate-guard apparently non-selective females in the wild (Huffard et al., 2008). Female octopuses copulate with multiple males in the wild (C. Huffard, pers. comm.) and in captivity (pers. obs.).

If the intensity of sperm competition is comparable throughout the family, male reproductive investment will be uniformly high. As male paternal investment in their offspring is considered to be limited to sperm, this investment should be visible as large testis size or abundant reproductive products, as has been documented among mammals, fishes and insects (Short, 1979; Kenagy & Trombulak, 1986; Stockley et al., 1997). Among cephalopods, such differences may be apparent in spermatophores.

Several aspects of octopodid spermatophores are remarkably consistent; the length of the sperm reservoir, the sperm-carrying part of the spermatophore, correlates very tightly with total spermatophore length across the family, except in species of the genus *Eledone*, which have a uniformly longer sperm reservoir (Voight, 2001). Spermatophore length shows limited variation, other than that due to body size (Voight, 2002; Allcock et al., 2008).

To determine if male allocation to reproduction differs among octopodid species, I tested for differences in the number of spermatophores that males of different species carry. Differences found may offer a predictive framework for future behavioral studies, as they may suggest differences in mating systems. Spermatophore count will be rated as informative if differences in the number of specimens considered are not linked to differences in counts, if species that share the possession of many or of few spermatophores are closely related, and if the few species known to have unusual reproductive behaviors also have abundant spermatophores. Apparently nonsensical variation in spermatophore number would give evidence that the number of spermatophores carried is uninformative for identifying unusual reproductive biology.

Octopodid Reproductive Biology

Male octopuses package sperm into spermatophores that they transfer to females via a modified arm, the hectocotylus (Wodinsky, 2008). Most information about how cephalopod spermatophores function derives from studies of Mann et al. (1970), summarized here. The long, thin spermatophores of octopuses contain two parts, the sperm reservoir and the ejaculatory apparatus. Spermatophore fluid is characterized by high molecular weight chemicals that generate a hyperosmotic fluid; the spermatophore's outer tunic functions as a semi-permeable membrane. Once a male's internal reproductive organs release a spermatophore and it contacts seawater, water is drawn inside the spermatophore. The increased internal pressure causes the ejaculatory apparatus of the spermatophore to extrude, presumably after it has been inserted into an oviduct (females have two). In time, the extruded ejaculatory apparatus balloons out to form the spermatophoric bladder; its eventual rupture releases sperm into the female's oviduct. The available data suggest that in octopodids, except species of *Eledone* detailed

below, and perhaps of *Vulcanoctopus*, sperm travels to spermathecae in the oviducal glands for storage. Insemination is thought to occur when mature eggs, released from the ovary, pass through the oviducal glands (Froesch & Marthy, 1975).

The reproductive anatomy of species of *Eledone* is nearly unique; males produce spermatophores with large sperm reservoirs (Voight, 2001) and females lack spermathecae (Perez et al., 1990). In South American species of *Eledone*, sperm penetrate ovarian eggs shortly after copulation, suggesting that the first male to copulate with a female is most likely to inseminate her eggs (Perez et al., 1990). The only known female specimen of *Vulcanoctopus hydrothermalis* González et al., 1998, also reportedly lacks spermathecae (González et al., 2008).

MATERIALS AND METHODS

Spermatophore Counts

The maximum number of spermatophores, and the longest mantle and spermatophore lengths were compiled from as many octopodid species as possible. The specimens examined are reported in Table 1, their depositories are: CAS = California Academy of Sciences; FMNH = Field Museum of Natural History; UA = University of Arizona collections. Because available specimens represented a narrow range of octopodid diversity, and not all specimens had intact spermatophores that could be both reliably measured and accurately counted, literature reports provided the second, and far more complete, data source (Table 1).

Use of maxima stresses the most comparable data (perhaps by comparing males with the lowest mating success), although it cannot assure full comparability. Different species are inevitably represented by different numbers of males that likely have attained different levels of maturity, and have had different reproductive histories. The number of spermatophores a male carries has been suggested to vary seasonally, especially if reproductive activity is seasonal, and with the male's recent sexual history (Mangold-Wirz, 1963). In octopodids, once begun, spermatophore production is thought to be continuous (Mangold, 1987), suggesting that an unmated, fully mature male will carry every spermatophore ever produced. However, as the capacity of the extensible storage (Needham's) sac is likely finite, some

spermatophores seemingly would have to be shed to accommodate those newly produced. Wodinsky (2008) supported this hypothesis by documenting that males release spermatophores in the absence of any known reproductive stimuli. When recognized, anomalous spermatophore counts, due for instance to occluded male reproductive organs (Wodinsky, 2008), were ignored. For example, Mann et al. (1970) reported a male of *Enteroctopus dofleini* (Wülker, 1910) had 32 spermatophores in its storage sac due to an occlusion; because this individual was considered aberrant, Pickford's (1964) report of 12 spermatophores was cited here as the maximum count for the species. Silva et al.'s (2002) plot of male size and spermatophore count among 2,333 males of *Octopus vulgaris* Cuvier, 1797, found the two showed no relationship; the two highest counts were 276 and near 190. The latter was selected as the more conservative and consistent with the report of 125 spermatophores for the species by Mangold-Wirz (1963). Although one of the 25 known male specimens of *Vulcanoctopus hydrothermalis* (González et al., 2002; pers. obs.) carried 114 spermatophores, the next highest value, 74, was cited here.

In total, data were compiled from 74 species, which are assigned to 13 groups (Table 1). Nine groups, *Abdopus*, *Amphioctopus*, *Bathypolypus*, the *Benthooctopus* clade as defined by Strugnell et al. (accepted), *Callistoctopus*, *Eleutheroctopus*, *Octopus* (*Octopus*) sensu Norman and Hochberg (2005a), *Scaevorgus* and the Southern Ocean endemic and deep-sea octopuses, defined as a clade by Strugnell et al. (in press; unpublished data), can be argued to be monophyletic. Our poor understanding of octopodid phylogeny (Voight, 1997) precludes assigning the remaining species to comparable groups. For instance, Norman & Hochberg's (2005a) restricted definition of *Octopus* left many species unplaced; they are here considered *Octopus* implicitly *sensu lato*. To simplify the presentation of the remaining 26 species, four artificial groups are created. These artificial groups are not based on hypothesized relationships, but serve only as a convenience to organize these species. One such group contains long-armed octopuses, a second contains species apparently restricted to shelf and slope depths from 100 to 600 m, the third includes species with maximum mantle lengths under 35 mm, analogous to the Pygmy octopuses of Norman & Sweeney (1997), and the fourth contains the remaining species.

Data Analyses

The median rather than the mean of number of spermatophores carried by the 74 species was used here because the data are not normally distributed. Species with at least two and half times the median number of spermatophores are arbitrarily defined as having abundant spermatophores. To test whether specimen availability affected the species' estimated number of spermatophores, the number of specimens representing species with more than the median number of spermatophores was compared to the number of specimens representing species with fewer than the median number of spermatophores using a Kolmogorov-Smirnov (K-S) test.

To describe the relationship between spermatophore count and body size, the maximum number of spermatophores for each species was plotted versus the longest mantle length reported for that species. A log-normal plot was generated to better illustrate the wide size range of species considered. Mantle lengths of males of *Callistoctopus aspilosomatus* (Norman, 1993a) that are "elongate" were not considered to ensure comparability.

To test whether abundant spermatophores tend to be comparatively short, log-transformed longest spermatophore length for each species was plotted versus the log of the longest mantle length for that species. Log-transformation equalizes measurement error over the wide size range considered, minimizes any error introduced by using back-calculated measurements or investigator bias, and minimizes the effect of outliers (Bookstein et al., 1985; Strauss, 1985). A regression line was generated based on all points. The distribution of points representing species with abundant spermatophores relative to the regression line (i.e., above, below) was compared to the expected 50:50 distribution about the line and tested with a G-test.

Behavioral Observations

Limited behavioral observations suggest differences exist in the potential for sperm competition among octopodids. Huffard et al. (2008) report mate guarding and sneaker males in *Abdopus aculeatus*. Multiple males have been observed in the wild attempting to copulate with lone females of *Octopus* (*O.*) *cyanea* Gray, 1849; *O.* (*O.*) *bimaculatus* Verrill, 1883 (Hanlon & Messenger, 1996; Tsuchiya & Uzu,

TABLE 1. Reported by group are each species included, its taxonomic authority, highest spermatophore count, number of male specimens considered, the longest mantle and spermatophore lengths in mm (ML; SpL) and the data source.

Taxon, Authority	Count	n	ML	SpL	Data source
<i>Abdopus</i> Norman & Finn, 2001; n = 9					
<i>A. horridus</i> (d’Orbigny, 1826)	70	10	30	12.8	Norman & Finn (2001)
<i>A. aculeatus</i> (d’Orbigny, 1834)	157	3	58	21	Norman & Finn (2001)
<i>A. abaculus</i> (Norman & Sweeney, 1997)	126	5	33	9.2	Norman & Sweeney (1997)
<i>A. capricornicus</i> (Norman & Finn, 2001)	135	3	43	13.5	Norman & Finn (2001)
<i>A. undulatus</i> Huffard, 2007	91	3	33.5	9.3	Huffard (2007)
<i>A. sp.</i> 2 (Philippines, Babuyan, Lubang Islands)	117	4	30	12.5	Norman & Finn (2001)
<i>A. sp.</i> A Gulf of Thailand	152	1	43	28	CAS 077999
<i>A. sp.</i> B Gulf of Thailand	157	1	58	15.6	CAS 078002
<i>A. sp.</i> of Ward Guam	160	8	35	12	Norman & Finn (2001)
<i>Amphioctopus</i> Fischer, 1882; n = 7					
<i>A. aegina</i> (Gray, 1849)	18	4	62	27	Norman & Sweeney (1997)
<i>A. arenicola</i> Huffard & Hochberg, 2005	11	3	51	37	Huffard & Hochberg (2005)
<i>A. burryi</i> (Voss, 1950)	11	1	47	23.6	Voss (1951)
<i>A. exannulatus</i> (Norman, 1993b)	8	1	50	62	Norman (1993b)
<i>A. mototi</i> (Norman, 1993b)	9	3	70	46.5	Norman (1993b)
<i>A. polyzenia</i> (Gray, 1849)	10	1	26	9	Norman (1993b)
<i>A. sp.</i> 1 Philippines, Visayan Sea	5	1	45	25	Norman & Sweeney (1997)
<i>Bathypolypus</i> Grimpe, 1921; n = 3					
<i>B. arcticus</i> (Prosch, 1849)	6	6	65	64	Muus (2002)
<i>B. bairdii</i> (Verrill, 1873)	6	6	90	72	Muus (2002)
<i>B. sponsalis</i> (Fischer & Fischer, 1892)	8	> 200	70	34	Mangold-Wirz (1963)
<i>Benthoctopus</i> clade (Strugnell et al., accepted); n = 8					
<i>Enteroctopus dofleini</i> (Wülker, 1910)	12	4	330	1130	Pickford (1964)
<i>E. magnificus</i> (Villanueva et al., 1991)	8	41	362	870	Villanueva et al. (1991)
<i>Benthoctopus johnsonianus</i> Allcock et al., 2006	19	1	113	104	Allcock et al. (2006)
<i>B. karubar</i> Norman et al., 1997	26	1	60	41	Norman et al. (1997)
<i>B. longibrachus</i> Ibáñez et al., 2006	31	12	115	70	Ibáñez et al. (2006)
<i>B. normani</i> (Massy, 1907)	25	9	100	119	Allcock et al. (2006)
<i>Benthoctopus</i> n. sp.	24	2	34	25.8	FMNH 278309
<i>Vulcanoctopus hydrothermalis</i> González et al., 1998	74	18	56.4	54	González et al. (2002)
<i>Callistoctopus</i> Taki, 1964; n = 6					
<i>C. nocturnus</i> (Norman & Sweeney, 1997)	1	1	60	28.2	Norman & Sweeney (1997)
<i>C. alpheus</i> (Norman, 1993a)	3	2	74	68.5	Norman (1993a)
<i>C. aspilosomatis</i> (Norman, 1993a)	2	3	45	39	Norman (1993a)
<i>C. dierythraeus</i> (Norman, 1993a)	4	1	135	102	Norman (1993a)

(continues)

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Taxon, Authority	Count	n	ML	SpL	Data source
<i>Callistoctopus graptus</i> (Norman, 1993a)	5	1	190	135	Norman (1993a)
<i>C. ornatus</i> (Gray, 1849)	9	2	104	51	Norman & Sweeney (1997)
<i>Eledone</i> Leach, 1817; n = 4					
<i>E. cirrhosa</i> (Lamarck, 1789)	290	50	110	54	Boyle & Knobloch (1984)
<i>E. gaucha</i> Haimovici, 1988	92	40	41	12.1	Haimovici (1988)
<i>E. massyae</i> Voss, 1964	60	111	80	30	Perez & Haimovici (1991)
<i>E. moschata</i> (Lamarck, 1789)	172	41	150	18.3	Akyol et al. (2007)
<i>Octopus</i> (<i>Octopus</i>) Cuvier, 1797; n = 3					
<i>O. (O.) vulgaris</i> Cuvier, 1797	190	2333	250	65	Silva et al. (2002)
<i>O. (O.) cyanea</i> Gray, 1849	> 300	ca. 15	160	48	Norman & Sweeney (1997)
<i>O. (O.) mimus</i> Gould, 1852	65	5	155	58	Guerra et al. (1999)
Southern Ocean endemic and deep-sea <i>Octopus</i> clade (Strugnell et al., in press; A. L. Allcock, pers. comm.); n = 4					
<i>Graneledone boreopacifica</i> Nesis, 1982	5	15	129	131	FMNH 278304
<i>G. gonzalezi</i> Guerra et al., 2000	5	5	84	100	Guerra et al. (2000)
<i>Pareledone felix</i> Allcock et al., 2007	6	8	42.4	70	Allcock et al. (2007)
<i>Vosseledone charrua</i> Palacio, 1978	3	1	53	56	Palacio (1987)
<i>Scaeurgus</i> Troschel, 1857; n = 4					
<i>S. unicolor</i> (Della Chiaje, 1839)	12	23	56	51	Mangold-Wirz (1963)
<i>S. tuber</i> Norman et al., 2005	8	5	17.8	12	Norman et al. (2005)
<i>S. juneau</i> Norman et al., 2005	8	4	35.7	36	Norman et al. (2005)
<i>S. neisis</i> Norman et al., 2005	7	3	47.3	30	Norman et al. (2005)
Long-armed octopuses; n = 3					
<i>Ameloctopus litoralis</i> Norman, 1992	70	2	30	6.4	Norman (1992)
<i>Thaumoctopus mimicus</i> Norman & Hochberg, 2005b	26	2	42.1	17	Norman & Hockberg (2005b)
<i>Wunderpus photogenicus</i> Hochberg et al., 2006	31	2	22	10.4	Hochberg et al. (2006)
Pygmy species (ML< 35 mm); n = 9					
<i>Octopus bocki</i> Adam, 1941	114	2	25	7	Norman & Sweeney (1997)
<i>O. fitchi</i> Berry, 1953	12	4	29	19	UA specimens
<i>Octopus gorgonus</i> Huffard, 2007	55	4	24	7.7	Huffard (2007)
<i>O. humilis</i> Huffard, 2007	30	1	26.4	17.5	Huffard (2007)
<i>O. mariles</i> Huffard, 2007	48	3	25	26.7	Huffard (2007)
<i>O. pumilus</i> Norman & Sweeney, 1997	110	5	31	7.8	Norman & Sweeney (1997)
<i>O. wolfi</i> (Wülker, 1913)	30	2	15	8.7	Norman & Sweeney (1997)
<i>O. sp.</i> 3 Philippines, Southern Negros, NW Mindanao Islands	68	2	21	9	Norman & Sweeney (1997)
<i>O. sp.</i> 5 Philippines, Cuyo Islands, S. negros and NW Mindanao; Cocos Keeling Islands	24	1	24	8.3	Norman & Sweeney (1997)
Miscellaneous species; n = 8					
<i>Cistopus indicus</i> (Rapp, 1835)	13	6	86	30	Norman & Sweeney (1997)

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Taxon, Authority	Count	n	ML	SpL	Data source
<i>Hapalochlaena fasciata</i> (Hoyle, 1886)	50	1	45	16	Tranter & Augustine (1973)
<i>Macroctopus maorum</i> (Hutton, 1880)	13	46	310	228	Grubert & Wadley (2000)
<i>Octopus filusus</i> Howell, 1867	90	38	37	10	Alvina (1965)
<i>O. laqueus</i> Kaneko & Kubodera, 2005	69	7	40.6	22	Kaneko & Kubodera (2005)
<i>O. cf. vitiensis</i> Hoyle, 1885	106	2	42	21.5	Norman & Sweeney (1997)
<i>O. sp.</i> 4 Philippines, Batan Islands	12	1	42	16.7	Norman & Sweeney (1997)
<i>Paroctopus digueti</i> (Perrier & Rochebrune, 1894)	25	15	42	22	UA specimens
Shelf and slope species (100 to 600 m depth); n = 6					
<i>Galeoctopus lateralis</i> Norman et al., 2004	8	9	30	33	Norman et al. (2004)
<i>Octopus micros</i> Norman, 2001	4	2	25	22.8	Norman (2001)
<i>O. palari</i> (Lu & Stranks, 1991)	1	1	54	25.1	Norman et al. (1997)
<i>O. pyrum</i> Norman et al., 1997	3	3	35	26	Norman et al. (1997)
<i>O. salutii</i> Verany, 1839	45	192	110	74	Mangold-Wirz (1963)
<i>Pteroctopus tetracirrhus</i> (Della Chiaje, 1830)	5	> 200	107	84	Mangold-Wirz (1963)

1997); *Vulcanoctopus hydrothermalis* (Rocha et al., 2002); and *O. kaurna* Stranks, 1990 (Norman, 2000). These species are predicted to have abundant spermatophores.

RESULTS

The maximum number of spermatophores carried by an individual male in the 74 octopodid species considered ranges from 1 to over 300 (Table 1); the median maximum number of spermatophores is 24. The number of specimens considered does not significantly differ between species with more than and with fewer than the median number of spermatophores ($D = 0.1622$, $P = 0.676$); the low value of the median cannot be attributed to sampling artifact.

The relationship between the number of spermatophores and log mantle length (Fig. 1) is not significant ($r = 0.072$). The relationship between number of spermatophores and log spermatophore length is statistically significant ($p < 0.05$, $df = 70$), but the predictive value of the relationship ($r^2 = 0.064$) is very low.

The 24 species with abundant (60 or more) spermatophores are not randomly distributed through the family (Fig. 1; Table 1). Sixteen of the 24 species are in one of three groups: *Abdopus*, *Eledone*, *Octopus* (*Octopus*). One member of the *Benthoctopus* clade, *V. hydro-*

thermalis, has abundant spermatophores, but other than *Enteroctopus*, the basal member of the group (Allcock et al., 2006), the remainder carry about the median number. Seven species with abundant spermatophores, *Ameloctopus litoralis* Norman, 1992; *O. filusus* Howell, 1867; *O. pumilus* Norman & Sweeney, 1997; *O. cf. vitiensis* Hoyle, 1885; *O. bocki* Adam, 1941; *O. sp.* 3 of Norman & Sweeney (1997); and *O. laqueus* Kaneko & Kubodera, 2005, remain very poorly known, both in terms of systematic relationships and general biology.

Nearly a quarter of the 24 species carrying nine or fewer spermatophores are members of *Callistoctopus*; eight are species known from shelf and slope depths (Table 1); four are members of the Southern Ocean endemic and deep-sea clade (Strugnell et al., in press, A. L. Allcock pers. comm.), and three are species of *Bathypolypus* and of *Amphioctopus*. *Octopus salutii* Verany, 1839; *Scaeurgus unichirrus* (Della Chiaje, 1839); and species of *Benthoctopus* are exceptional among deeper-water species in carrying from 12 to 45 spermatophores (Table 1).

Maximum spermatophore length generally increases with maximum mantle length among the 74 octopodid species considered ($r^2 = 0.716$; Fig. 2). In species with abundant spermatophores, spermatophore lengths are significantly more likely to fall low on plots of log

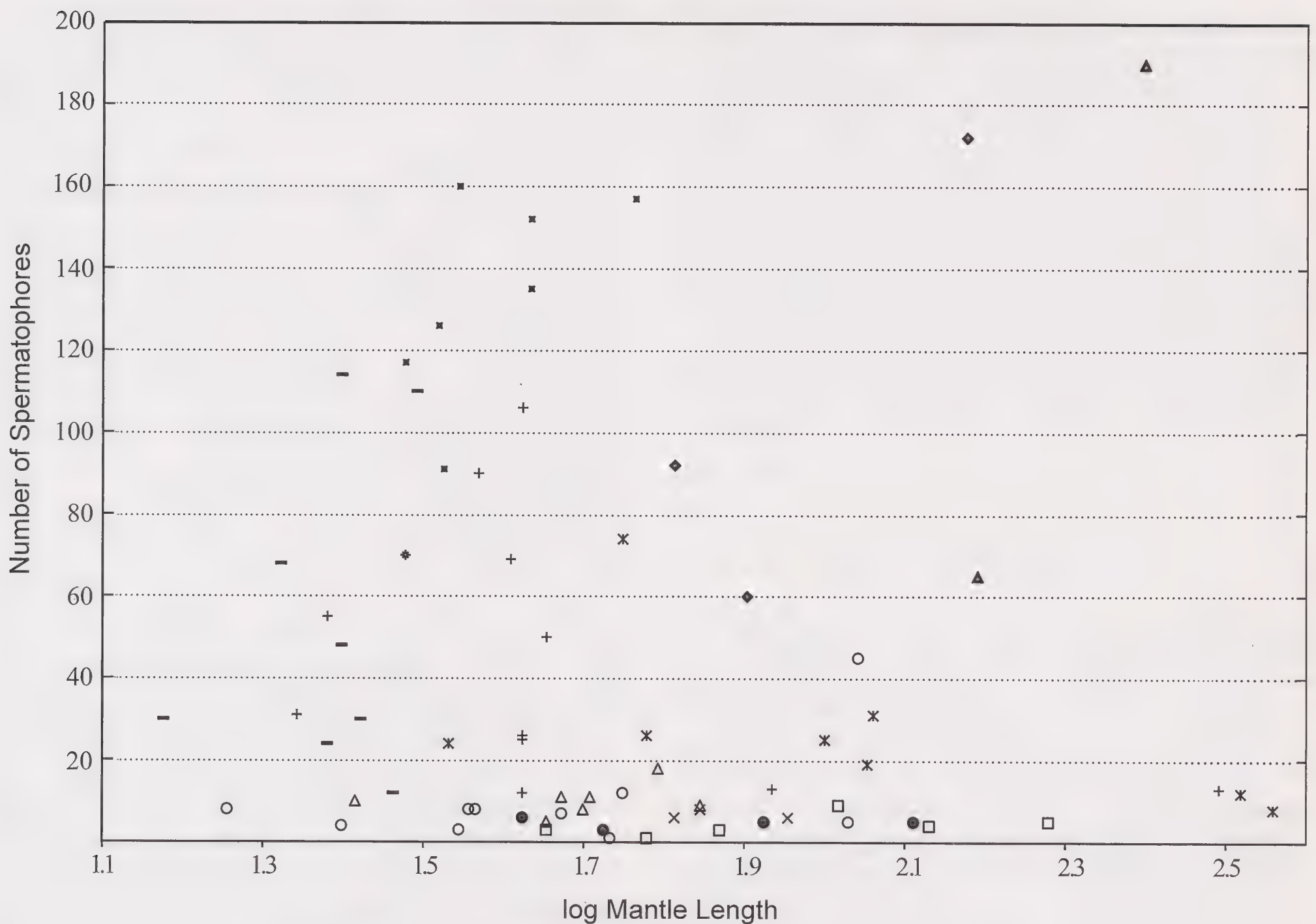


FIG. 1. Plotted is the maximum number of spermatophores carried by an individual male versus the longest log-transformed mantle length for 72 of the 74 species considered. Excluded are *Octopus cyanea* in which the count exceeds 300 spermatophores (Norman & Sweeney, 1997) and *Eledone cirrhosa* in which the count reaches 290 (Boyle & Knobloch, 1984). Groups with abundant spermatophores are *Eledone* (solid diamond), *Abdopus* (solid square), *O. (Octopus)* (solid triangle). Groups from low temperatures are *Bathypolypus* (X), the *Benthoctopus* clade (X with a vertical line), South Ocean endemic and Deep-sea clade (solid circle) and Shelf and slope species, including *Scaevargus* (open circle). Species of *Amphioctopus* are represented by open triangles, those of *Callistoctopus* by open squares, and the artificial groups of pygmy species by bars and long-armed and miscellaneous species by +.

spermatophore length versus log mantle length (Fig. 2) than in species with fewer spermatophores ($G = 6.2$; $P < 0.025$). *Vulcanoctopus hydrothermalis*, a robust specimen of *Abdopus* sp. A (CAS 077999), *O. laqueus*, and *O. cf. vitiensis* are exceptional in having abundant spermatophores that are at least as long as predicted by the regression line calculated for all 74 species (Fig. 2).

DISCUSSION

Sixteen of the 24 species identified here as having abundant spermatophores, arbitrarily defined as 60, or two and a half times the median, are members of three groups, *Abdopus*, *Eledone* and *Octopus (Octopus)*. A quarter of

the species with nine or fewer spermatophores are members of the genus *Callistoctopus*. Others are all species of *Bathypolypus* included and the Southern Ocean endemic and deep-sea clade containing *Graneledone*, *Pareledone*, and *Vosseledone* (Strugnell et al., in press; J. Strugnell & L. Allcock, pers. comm.). The hypothesis that related species share spermatophore counts is supported, although seven other species with abundant spermatophores – *Ameloctopus litoralis*, three of eight pygmy species, and three miscellaneous species – can currently only be assigned to groups of convenience. Given that spermatophore counts are not impacted by differences in the number of specimens considered, are abundant spermatophores associated with unusual reproductive patterns?

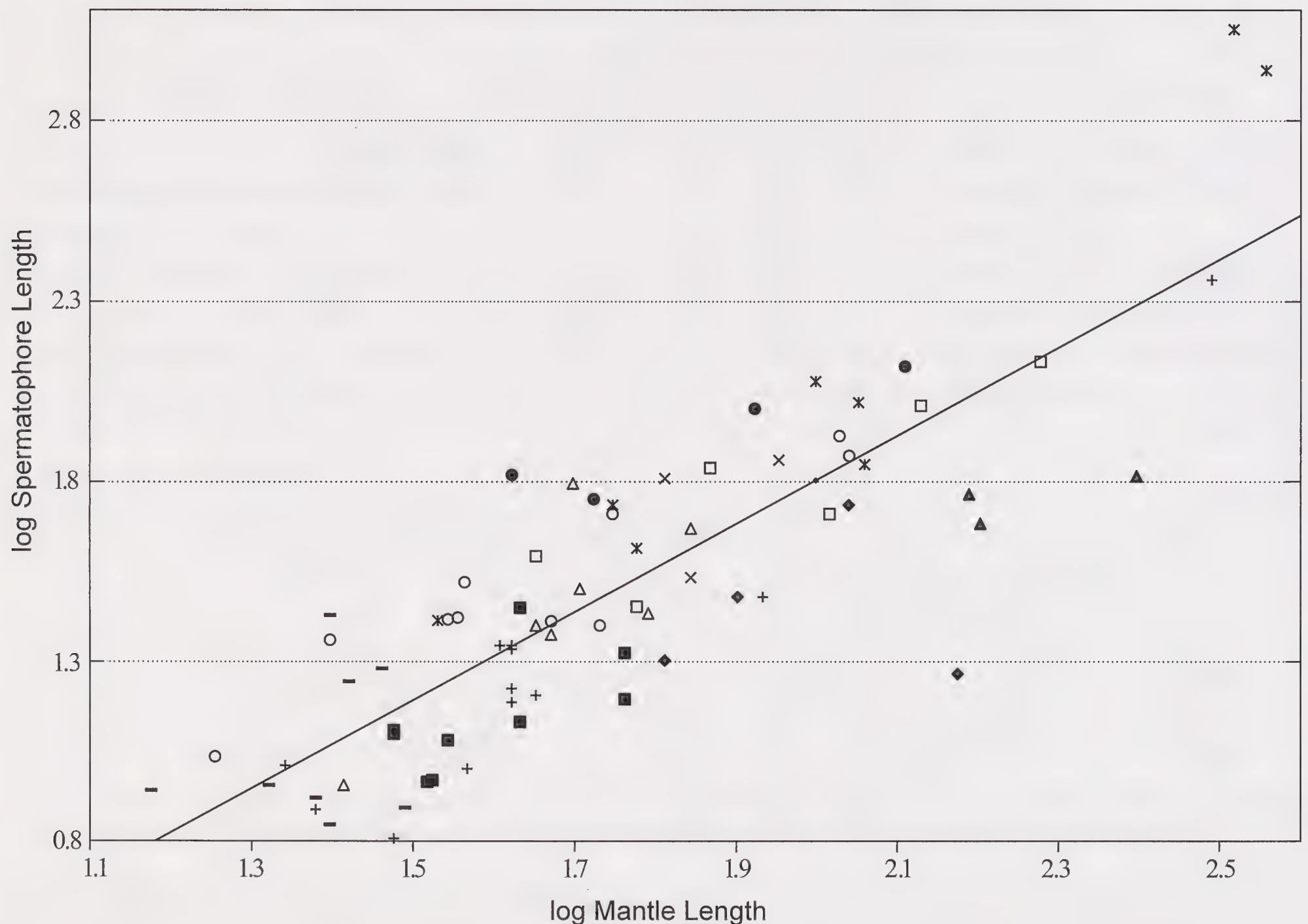


FIG. 2. Plotted are log-transformed spermatophore length versus log-transformed mantle length for the 74 species considered here. The line is the relationship of spermatophore and mantle length for all species considered ($y = 1.22x - 0.646$). Groups with abundant spermatophores are *Eledone* (solid diamond), *Abdopus* (solid square), *O. (Octopus)* (solid triangle). Groups from low temperatures are *Bathypolypus* (X), the *Benthoctopus* clade (X with a vertical line), South Ocean endemic and Deep-sea clade (solid circle) and Shelf and slope species including *Scaeurgus* (open circle). Species of *Amphioctopus* are represented by open triangles, those of *Callistoctopus* by open squares, and the artificial groups of pygmy species by bars, and long-armed and miscellaneous species by +. The two exceptionally large spermatophores are those of *Enteroctopus* species.

The presence of abundant spermatophores in *Eledone* is consistent with their known reproductive biology. As discussed above, males of *Eledone* that copulate with the greatest number of virgin females likely sire the most offspring (Perez et al., 1990). Having abundant spermatophores may be vitally important to increasing male fitness, especially in dense, reproductively synchronized populations of *Eledone*. The only known female specimen of *Vulcanoctopus hydrothermalis* also reportedly lacks spermathecae (González et al., 2008), and multiple males have been reported to grapple with a lone female (Rocha et al., 2002). Both the lack of spermathecae and multiple mates are predicted to select for an increase in the number of spermatophores available.

As summarized above, among species in which eggs are assumed to be inseminated as

they pass through the oviduct at spawning, at least two males have been reported attempting to copulate with a lone female of *A. aculeatus*, *O. (O.) cyanea*, and *O. (O.) bimaculatus*, all species with abundant spermatophores, as predicted by the observed mating. Males also appear to grapple over females in *O. karna* (Norman, 2000); spermatophores in that species are predicted to be abundant.

Also deserving attention are species that appear to carry very few spermatophores, notably those of *Callistoctopus*. All six members of this genus included here carry nine or fewer spermatophores, however, each of these species is represented by fewer than ten specimens (Table 1). Although too few specimens are available for robust statistical tests, among the 30 other species that are represented by so few males, only 26.6% carry so few sper-

matophores. The few spermatophores carried by males of *Callistoctopus* may therefore reflect a real difference in the reproductive biology of the group.

Deep-sea octopuses are considered to have a few, large spermatophores as an adaptation to the deep sea (Voss, 1988), as shown by *Bathypolypus* and the Southern Ocean endemic and deep-sea clade of Strugnell et al. (in press). Species of *Benthooctopus* included here (Table 1), however, carry at least the median number of spermatophores for the family as a whole. Spermatophores of octopuses that live in cold temperatures are larger than predicted by mantle length (Fig. 2), perhaps because low temperatures increase cell and egg size, as occurs in ectotherms of diverse phyla (Van Voorhies, 1996). Although the pattern is yet to be experimentally demonstrated in cephalopods, both the squid *Loligo gahi* Orbigny, 1835 (Arkhipkin et al., 2000), and the octopodid *Eledone cirrhosa* (Lamarck, 1789) (Boyle et al., 1988) produce larger eggs and spermatophores in cold water than do conspecifics in warmer waters, supporting this hypothesis.

Spermatophore counts appear to be independent of differences in specimen availability and to vary within narrow limits among closely related species. A necessary simplifying assumption of this study is that all spermatophores are created equal, regardless of apparent differences in sperm mass thickness or coiling. Our knowledge of these differences, and of the chemistry of spermatophore fluid, must be increased to test the generality of that assumption.

Although it has received minimal attention, spermatophore width is a very important variable. A key determinant of volume, width may also affect the rate of the spermatophore reaction. If abundant spermatophores are not only shorter, but thinner, as limited data suggest, their greater surface area would accelerate the movement of water into the spermatophore and speed the spermatophoric reaction. Faster spermatophore reactions might be important where male competition is intense or matings are brief or occur while foraging. When a spermatophore is inserted into an oviduct, and presumably held by the muscular oviduct, the outer casing can dangle free. Removing the spermatophore before the spermatophoric reaction is complete, that is before the sperm bladder is free of the outer casing, would result in loss of the sperm. Faster reactions, as maybe achieved with thinner spermatophores, might minimize this risk. That males attempt to remove partially everted spermatophores seems

more likely than suggestions (Norman, 2000; Norman et al., 2004) that males scrape sperm from the female's oviducts with their ligula, or that in *Galeoctopus lateralis* Norman et al., 2004, use their ligula to rupture the "outer casings of the sperm bulbs of previous suitors".

Regardless, octopodid reproductive biology likely contains far greater diversity than has been expected. Behavioral observations are required to test whether the species with unusual spermatophore counts have unusual reproductive behaviors, but count data appear to offer a framework on which predictions can be made.

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